

Effect of a standardized extract of red orange juice on proliferation of human prostate cells in vitro

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Abstract

A standardized extract of red orange juice (ROE) was shown to inhibit proliferation of fibroblast and epithelial prostate cells. These data suggest that the antiproliferative properties of ROE cannot be ascribed to cytotoxic effect and highlight its potential usefulness in the management of benign prostatic hyperplasia.

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1. Introduction

Benign prostatic hyperplasia (BPH) is an endocrine disorder causing excessive cellular proliferation of epithelial and stromal elements [1]. Moreover, growth factors may play a role in the mechanism of normal and pathological prostate development [2]. Tenniswood [3] suggested that BPH development might be caused by inappropriate expression of some growth factors. Basic fibroblast growth factor was the first one to be isolated in BPH; it seems to be localized in the prostatic fibroblasts and its level is increased in BPH in comparison with normal tissue [4].

Herbal preparations are widely used for medical treatment of BPH [5]. In the present study we investigated the effect of a standardized extract of red orange juice (ROE) on the growth of epithelial prostatic cells and fibroblasts.

2. Experimental

2.1. Plants material

Is a standardized extract obtained from the juice of three pigmented varieties of *Citrus sinensis* (Moro, Tarocco, Sanguinello) through the following process. Briefly, the juice was filtered using 0.2 μm paper filter in order to remove any impurities and then was applied to an XAD-16 column (Rohm and Haas, Philadelphia, PA, USA). The resins were eluted with an ethanol/water solution (50:50) then ethanol was removed by evaporation and the aqueous residue was spray-dried. It contains cyanidin glycosides (3.27%), hydroxycinnamic acids (2.3%) and flavanone glycosides (0.741%). *Serenoa repens* extract (Indena S.p.A. Milan, Italy) is a standardized ethanolic extract containing 88.1% fatty acids, β -sitosterol, campesterol, stigmasterol, flavonoids and hexacosanol.

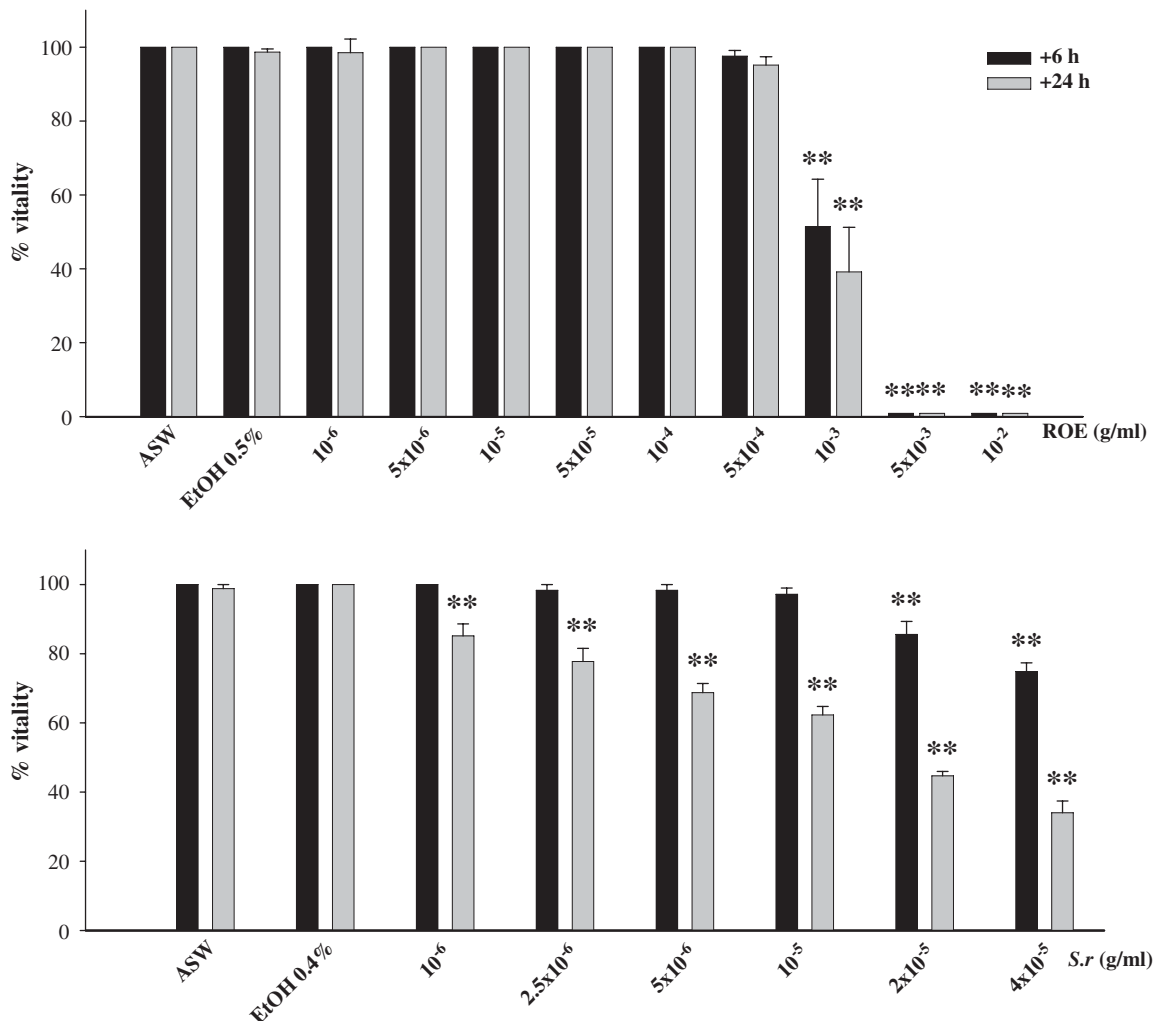


Fig. 1. Effect of ROE and ethanolic extract of *Serenoa repens* (*S.r.*) on brine shrimp lethality bioassay. * $P < 0.05$, ** $P < 0.01$ vs ASW (artificial sea water), Mann–Whitney Rank Sum Test.

2.2. Brine shrimp lethality bioassay

The *Artemia salina* assay was performed according to Meyer et al. [6], with light modifications. About ten-fifteen shrimps (100 μ l) were transferred into each well, in 24 well plates, and added with different concentrations of ROE (from 10^{-6} to 10^{-2} g/ml) or *S. repens* extract (from 10^{-6} to 4×10^{-5} g/ml). The plates were maintained under illumination, at 25–30 °C. The nauplii were counted by means of a 10 \times binocular microscope after 6 and 24 h, counting viable vs non-viable shrimps in each well. Ethanol (200 μ l) was used as vehicle. Results are reported as percent of vitality.

2.3. Evaluation of cell proliferation and cytotoxicity

The effect of extracts was evaluated on a normal human prostatic epithelial cell line (PZ-HPV-7; ATCC: CRL-2221) and on a lung fibroblast cell line of Chinese hamsters (V79-4; ATCC: CCL-93). Cell lines were grown in media as recommended by the suppliers.

PZ-HPV-7 cells (5×10^4 cells/well; in 24 well plates) and V79-4 cells (8×10^3 cells/well; in 96 well plates) were cultured for 48 and 24 h, respectively. Different concentrations of ROE or *S. repens* extract were added. Cell cytotoxicity was measured using the Trypan blue exclusion assay [7]. Cell proliferation was measured by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenil tetrazolium bromide] (MTT) colorimetric assay [8,9]. Each experiment was carried out in triplicate and repeated at least twice.

2.4. Statistical analysis

Statistical analysis was performed by: Mann–Whitney Rank Sum Test (Brine Shrimp Lethality Bioassay), Dunn's Method (Cell Culture). A $P < 0.05$ was considered statistically different.

3. Results and discussion

In the brine shrimp lethality bioassay, *S. repens* ethanolic extract resulted toxic beginning from 2×10^{-5} g/ml at 6 h, and beginning from 10^{-6} g/ml at 24 h. ROE resulted toxic beginning from 10^{-3} g/ml at 6 and 24 h. These results indicate that in this bioassay both ROE and *S. repens* ethanolic extract have a very low toxicity (Fig. 1).

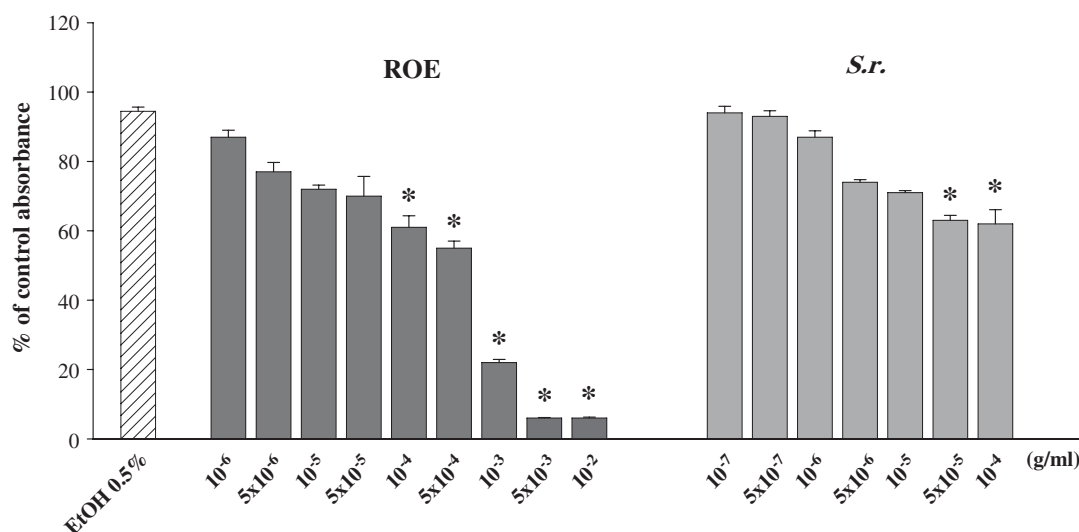


Fig. 2. Effect of ROE and ethanolic extract of *Serenoa repens* (S.r.) on proliferation of V79-4 cells, measured by MTT assay after 24 h of exposure. Data are reported as mean value \pm SEM of six wells for every dose level. * $P < 0.05$, Dunn's method.

In the MTT assay on V79-4 cells, *S. repens* ethanolic extract and ROE induced a significant antiproliferative effect at the higher concentration employed (5×10^{-5} and 10^{-4} g/ml, respectively) (Fig. 2). Similar results were obtained on PZ-HPV-7 cells; the inhibition of proliferation was still present after 48 h of exposure (Fig. 3).

In the Trypan blue exclusion assay, *S. repens* ethanolic extract did not show a significant cytotoxic effect, while ROE appeared toxic from 5×10^{-3} g/ml (Fig. 4).

These results indicate that effects observed in MTT assay with *S. repens* ethanolic extract and ROE (lower concentrations) are not attributable to cytotoxicity; on the other hand, the effect of exposure to higher concentrations of ROE is partially due to its cytotoxicity.

Cell proliferation and apoptosis are physiological mechanisms involved in the maintenance of prostate integrity and function. These processes are under the control of intrinsic and extrinsic factors, such as growth factors and hormone levels. In normal adult prostate, the mean apoptotic and proliferative indices are low, indicating a balance between these processes. In contrast, BPH tissue shows an imbalance in the two processes in favour of cell proliferation. Our present findings confirm that *S. repens* extract inhibits prostate cell proliferation and highlight the potential usefulness of ROE, even if less active in the management of BPH. Further investigations are warranted to clarify its mechanism of action.

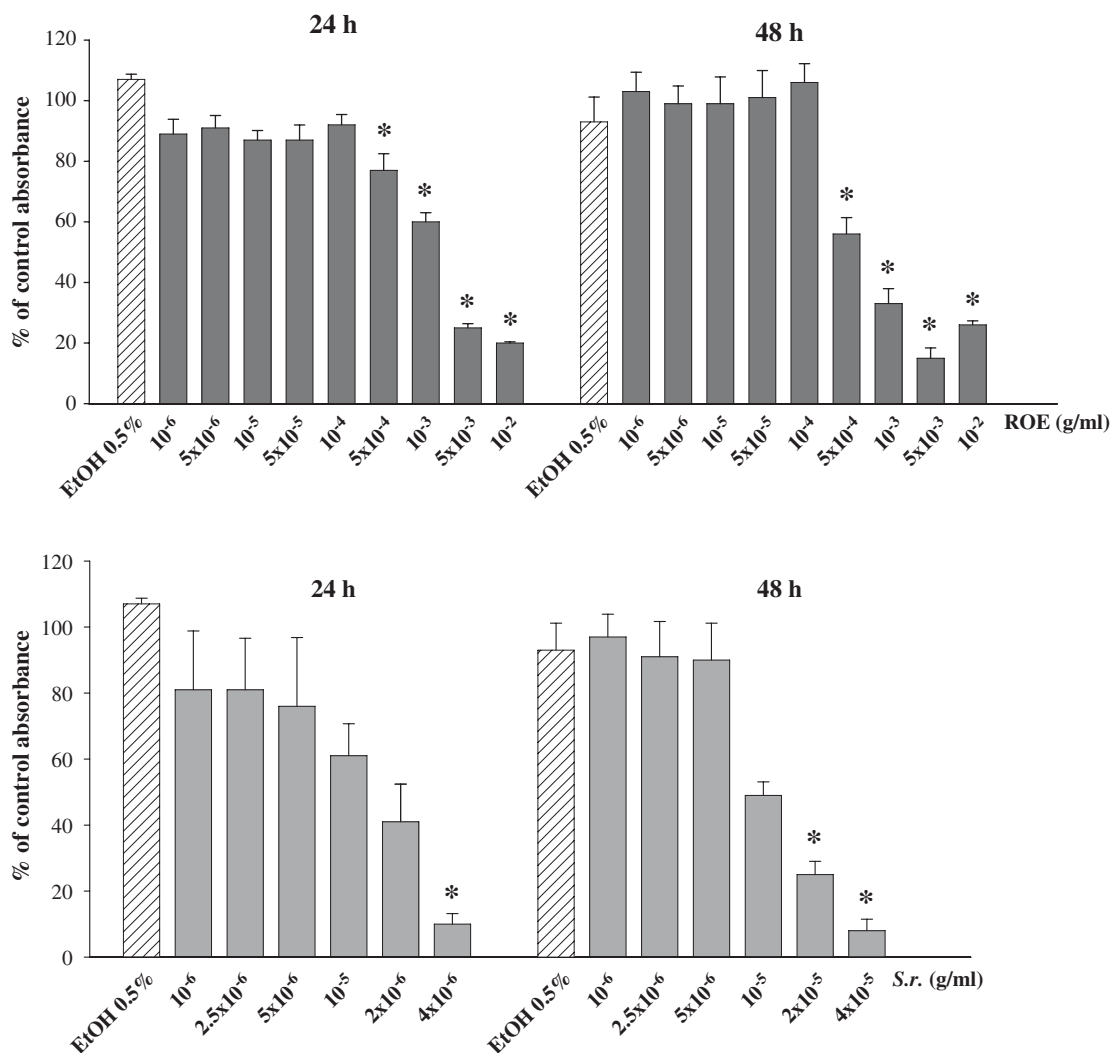


Fig. 3. Effect of ROE and ethanolic extract of *Serenoa repens* (S.r.) on proliferation of PZ-HPV-7 cells, measured by MTT assay after 24 and 48 h of exposure. Data are reported as mean value \pm SEM of six wells for each dose level. * $P < 0.05$, Dunn's test.

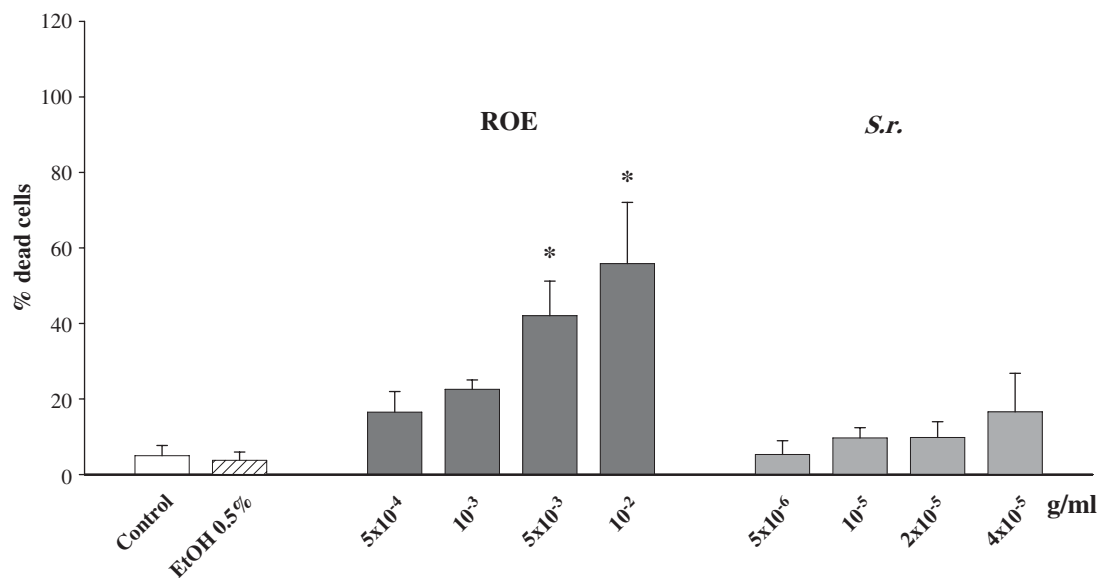


Fig. 4. Effect of ROE and ethanolic extract of *Serenoa repens* (*S.r.*) on proliferation of PZ-HPV-7 cells (24 h of incubation) measured by Trypan blue exclusion assay. Data represent the mean \pm SEM. * $P < 0.05$, Dunn's method.

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